REMARKS

Claim 21 has been canceled.

Claims 1 and 2 have been amended to include the step (c) of separating said PGCs cultured *in vitro* for at least 10 days from a culture medium without employing the procedure of Ficoll density gradient centrifugation. The basis of this amendment is on line 23 of page 8 - line 7 of page 9, lines 20 and 21 of page 12, and line 22 of page 15 – line 4 of page 16. No new matter is added.

In addition, claims 1 and 2 have been amended to recite the efficiency of germline transmission of the PGCs injected to be between 44.7% and 49.7% in the body of the claims. Support for this amendment can be found in TABLE II on page 15, and line 22 of page 15 - line 4 of page 15. Hence, no new matter is added.

The preambles and certain expressions of claims 1 and 2 have been more clearly revised in accordance with the Examiner's recommendations.

Furthermore, expressions pointed out by the Examiner in claims 18 -20 have been amended to be clear.

In claim 2, the "recipient embryo" has been limited to the "recipient chicken embryo."

The preceding amendments and the following remarks are believed to be fully responsive to the outstanding Office Action and are believed to place the application in condition for allowance.

The Examiner is respectfully requested to reconsider and withdraw the rejections in view of the amendments and remarks as set forth herein.

I. Claim Objections

In order to overcome the claim objections, the Applicants have amended claims as follows.

The preambles of claims 1 and 2 have been amended to recite a method of improving the ability to prepare germline chimeric chickens, and a method of improving germline transmission when preparing a chicken germline chimera, respectively, in accordance with the Examiner's suggestions.

In addition, the amount of the efficiency of germline transmission of PGCs and the efficiency of making germline chimeras has been amended to be between 44.7% and 49.7% in the body of claims 1 and 2.

The phrase "whereby the chicken germline chimera is prepared" has been replaced with "such that a chicken germline chimera occurs."

In claims 1 and 2, the phrase "injecting cultured PGCs into the recipient embryo is carried out by injecting cultured PGCs into the dorsal aorta of a recipient chicken embryo" described at the end of claim has been incorporated into the injecting step (d) as "injecting said separated PGCs into the dorsal aorta of a recipient chicken embryo."

In claim 18, the phrase "the source of the chicken PGCs" has been replaced with the "the chicken embryonic gonad."

In claim 19, the "recipient embryo" has been limited to the "recipient chicken embryo."

In claim 20, the expression "the source of the chicken PGCs" has been replaced with the "the chicken embryonic gonad" and the phrase "the source of the chicken embryo" has been replaced with "the recipient chicken embryo."

The Applicants believe that the claim objections should be overcome by the above

amendments. Therefore, the Applicants respectfully request that these objections relating to definiteness of the claims be withdrawn.

II. Claim Rejections under 35 USC § 112

The Examiner rejected claims 1, 2, 8-11, and 18-21 on the basis that the present specification does not provide enablement for injection of PGCs into any embryo. Claims 18-21 have been also rejected because the scope of recipient embryo is too broad.

In order to overcome the rejections, the Applicants have amended claims 1, 2, and 19 by limiting the recipient embryo to the recipient chicken embryo.

Accordingly, the Applicants respectfully request that this rejection be withdrawn.

III. Indefiniteness under 35 USC § 112

The Examiner has pointed out that claim 1 is indefinite, because claim 1 substantially refers to a method of improving the ability to make germline chimeric chickens, not merely improving the germline transmission efficiency of PGCs as claimed.

In order to overcome the rejection, the Applicants have amended claim 1 by incorporating the phrase "a method of improving the ability to prepare germline chimeric chickens" into the preamble.

Accordingly, the Applicants respectfully request that this rejection be withdrawn.

The Examiner has rejected claims 1 and 2 for indefiniteness, on the basis that the expression "an improved germline transmission efficiency of up to 49.7%" is unclear.

In order to overcome the rejection, claims 1 and 2 have been amended to recite the efficiency

of germline transmission of the PGCs injected to be between 44.7% and 49.7%. Support for this amendment can be found in TABLE II on page 15 and line 22 of page 15 - line 4 of page 15. By this amendment, it have been clarified that the efficiency of germline transmission of the PGCs is in the range of 44.7-49.7%.

Hence, the Applicants respectfully request that this rejection be withdrawn.

The Examiner has also pointed out that the phrase "said PGCs in vitro cultured in step (b) express a stage specific embryonic antigen-1 (SSEA-1)" in claims 1 and 2 lacks antecedent basis. In order to overcome this rejection, the Applicants amended claims 1 and 2 by replacing the indicated phrase "said PGCs in vitro cultured in step (b) express SSEA-1" with "said PGCs are positive for SSEA-1 after in vitro culture for at least 10 days." By this amendment, it is clearly understood that the PGCs become to be positive for SSEA-1 after it was cultured in vitro for at least 10 days.

The Examiner also indicated that the phrase in claims 1 and 2 regarding "injecting the cultured PGCs into the recipient embryo" is indefinite because the meaning of the "cultured PGCs" is not clear.

In the presently amended claims 1 and 2, the step (c) of the separation process of PGCs which have been cultured in vitro for at least 10 days is incorporated and it is described in the step (d) that the PGCs separated according to the step (c) are injected into the recipient chicken embryo. Accordingly, claims 1 and 2 have been amended to be clearly state that the PGCs, which are cultured in vitro for at least 10 days and separated without using the Ficoll density gradient centrifugation, are injected into the recipient chicken embryo. By these amendments, the rejection should be overcome.

In addition, claims 1 and 2 have been amended by moving the limitation of culturing the PGCs with gonadal stromal feeder cell layer into the part describing the culturing step (b).

Furthermore, claim 21 indicated as being indefinite has been canceled.

Therefore, the Applicants respectfully request that these rejections be withdrawn.

IV. Claim Rejections under 35 USC §102

The Examiner rejected claims 1, 2, and 8-11 as being anticipated by Han (Theriogenology, Nov. 2002, Vol. 58, pg 1531-1539).

The Applicants have amended claims 1 and 2 by incorporating the step of (c) separating said PGCs cultured in vitro for at least 10 days from a culture medium without employing the process of Ficoll density gradient centrifugation.

The amended instant claims have the characteristics of i) separating PGCs cultured *in vitro* for at least 10 days from a culture medium without employing the process of Ficoll density gradient centrifugation and ii) the efficiency of germline transmission of the PGCs injected is between 44.7% and 49.7%.

The basis of the amendment can be found on line 23 of page 8 – line 7 of page 9, lines 20 and 21 of page 12, and line 22 of page 15 – line 4 of page 16. Thus, no new matter is added.

[Line 23 of page 8 – line 7 of page 9 of the specification]

It has generally accepted that Ficoll gradient separation contributes to increasing the population ratio of PGCs for effective injection into the recipient embryo (14). Several previous reports showed that the positive effect of Ficoll treatment on the separation of PGCs and its safety on maintaining cell viability. However, the data in Examples described below clearly demonstrated that except for the transplantation of 0 day-cultured gPGCs, no beneficial effect of Ficoll separation on the germline

transmission efficiency of PGCs was found in our developed system. On the contrary, Ficoll separation negatively affected the germline transmission in the present method. Accordingly, it could be recognized that the present invention could completely avoid the need for Ficoll treatment, enabling the production of avian chimeras or transgenics to become simplified and feasible.

[Lines 20 and 21 of page 12]

5) culture for 10 days then transfer without Ficoll treatment

[Line 22 of page 15 – line 4 of page 16]

Within the same in vitro culture duration, improved efficacy of germline transmission was found in no Ficoll-treated group, compared with Ficoll-treated group. Such effect was prominent in the groups of 10-day culture; significant ($p<0.0001$) increase in germline transmissionn, regardless of chimera sexuality, was found (44.7 to 49.7% vs. 10.7% to 25.4%).

[REFERENCES]

14. Yasuda Y, Tajima A, Fujimoto T, Kuwana T. A method to obtain avian germ-line chimeras using isolated primordial germ cells. J Reprod Fertil 1992; 96: 521-528.

Meanwhile, in view of the disclosure of the reference 14 (Yasuda Y, et al., A method to obtain avian germ-line chimeras using isolated primordial germ cells, J Reprod Fertil 1992; 96:521-528), which has been incorporated into the present specification as a reference, it is clearly understood that terms "Ficoll gradient separation" and "Ficoll treatment," which are used in this specification mean a separating and concentrating process of PGCs from a culture media using the Ficoll density gradient centrifugation.

As shown in the data of Table II on page 15 of the present specification, it has been demonstrated that the efficacy of germline transmission was significantly improved in the 10-days culture and Ficoll-non treated group compared with the 10-days culture and Ficoll-treated group (44.7 to 49.7% vs. 10.7 to 25.4%). From this result, it can be easily understood that Ficoll

separation negatively affects the germline transmission efficiency in the present method. Thus, the Applicants have amended claims 1 and 2 by incorporating the separation step that does not include the Ficoll density gradient centrifugation procedure and more strictly limiting the efficiency of germline transmission of the PGCs injected to 44.7 - 49.7%. It is noteworthy that the present invention could completely avoid the need for separation step using Ficoll gradient centrifugation, enabling the production of avian chimeras or transgenics to become simplified and feasible.

On the contrary, Han (Theriogenology, Nov. 2002, Vol. 58, pg 1531-1539) does not show any concrete comparative evidence that the method not including the Ficoll gradient centrifugation is more effective than the method having the Ficoll gradient centrifugation. More specifically, Han does not provide any data verifying the improvement of germline transmission efficiency in the method of preparing germline chimeras where not employing the PGCs separation procedure using Ficoll density gradient centrifugation. Attention should be paid to the fact that the germline chimerism efficiency is 21% by the Han's method (see the description on page 1536, lines 19-21 and Table 1 of Han's paper). It is also very noticeable that the 21% of germline transmission efficiency is the value which corresponds to that of the Ficoll-treated 10 days culture group in the present invention (see the germline transmission efficiency of 10.7-25.4% in Table II on page 15 of the present specification).

Accordingly, the method described in Han's paper is strongly presumed to be one that includes the PGCs separation procedure via Ficoll density gradient centrifugation. Based on this, the Applicants traverse the Examiner's assertion that the method of Han inherently resulted in germline transmission efficiency of 49.7%.

It is clearly evident that the technical features of the present method that the Ficoll-gradient separation procedure is not employed and the germline transmission efficiency is limited to 44.7-49.7% are not disclosed in the Han's paper.

Consequently, the Applicants respectfully request that these rejections be withdrawn.

V. Claim Rejections under 35 USC § 103

The Examiner rejected claims 1, 2, 8-11, and 18-21 as being unpatentable over Kim (Transgenic Research, February 2002, Vol. 11, No. 1, pp. 85; presented to the public at the Transgenic Animal Research Conference. Tahoe City, California, USA. September 09-13, 2001) in view of Chang (Cell Biology International, 1997, Vol. 21, No. 8, pg 495-499), Zandong (Transgenic Research, February 2002, Vol. 11, No. 1, pp. 85; presented to the public at the Transgenic Animal Research Conference. Tahoe City, California, USA. September 09-13, 2001) and Han (Theriogenology, Nov. 2002, Vol. 58, pg 1531-1539).

The presently amended claims 1 and 2 include characteristics of i) separating PGCs cultured *in vitro* for at least 10 days from a culture medium without employing the process of Ficoll density gradient centrifugation and ii) the efficiency of germline transmission of the PGCs injected is between 44.7% and 49.7%.

The reference of Kim (Transgenic Research, February 2002, Vol. 11, No. 1, pp. 85; presented to the public at the Transgenic Animal Research Conference. Tahoe City, California, USA. September 09-13, 2001) does not disclose the step of separating PGCs from a culture media that does not use the procedure of Ficoll density gradient centrifugation. In addition, please note that in the reference of Kim the germline transmission efficiency is described to be *25.6%* on average

(see line 4 from the below). Accordingly, it is not suggested or taught that the efficiency of germline transmission is between 44.7% and 49.7% by the reference of Kim.

The reference of Chang (Cell Biology International, 1997, Vol. 21, No. 8, pg 495-499) also does not suggest or teach that the method comprising the step of separating PGCs from a culture media not employing the procedure of Ficoll density gradient centrifugation. In addition, please also note that in the reference of Chang the frequency of germline transmission of donor PGCs is described to be *1.3-3.1%* (see the RESULTS and Table 3). Therefore, it is clear that Chang's method does not result in the gremline transmission efficiency of 44.7 - 49.7%.

The reference of Zandong (Transgenic Research, February 2002, Vol. 11, No. 1, pp. 85; presented to the public at the Transgenic Animal Research Conference. Tahoe City, California, USA. September 09-13, 2001) also does not disclose the method for preparing germline chimeric chicken including the step of separating PGCs from a culture media without utilizing the procedure of Ficoll density gradient centrifugation. Furthermore, there is no evidence that the germline transmission efficiency will be in the range of 44.7-49.7% when it is performed in accordance with the method disclosed in the reference of Zandong.

As mentioned above, in the reference of Han (Theriogenology, Nov. 2002, Vol. 58, pg 1531-1539), it is clearly described that the efficiency of chimerism is 21%, which is in the range of Ficoll treated group of the present invention. In addition, there is no suggestion or teaching that the method for preparing germline chimerism comprising the step of separating PGCs from the culture media which does not employ the procedure of Ficoll density gradient centrifugation.

As our review on the references, the references indicated by the Examiner do not disclose the technical features of the present invention. Hence, contrary to the Examiner's allegation, a

skilled person would have had no reasonable expectation that PGCs originated from embryonic gonad, cultured for at least 10 days and separated from a media not using the process of Ficoll gradient centrifugation could successfully used for improving germline transmission of 44.7-49.7%, as recited in the present claims.

We would therefore ask that the rejection for obviousness be withdrawn.

CONCLUSION

In order to overcome the claim objections, the preamble of claims 1 and 2 are amended as suggested by the Examiner, and the efficiency of germline transmission has been described in the body. In addition, claims 1, 2, and 18-20 have been amended to be clear by revising certain expressions that have been indicated as unclear.

With regard to the rejection concerning the breadth of avian, this rejection has been overcome by the amendment of limiting the embryo to the chicken embryo.

The rejection of indefiniteness of claims 1 also has been overcome by reciting more clear expression as recommended by the Examiner.

The indefiniteness rejection of claims 1 and 2 concerning to the expression of "an improved germline transmission efficiency of up to 49.7%" has been overcome by the amendment of replacing with more clear efficiency range "44.7-49.7%."

Furthermore, in claims 1 and 2, the phrase "wherein said PGCs in vitro cultured in step (b) express SSEA-1" has been replaced with "said PGCs are positive for SSEA-1." In addition, the phrase regarding "injecting the cultured PGCs" is substituted with the expression "injecting said PGCs cultured in vitro for at least 10 days."

The rejection regarding the indefiniteness of claim 21 has been overcome by the amendment of canceling of claim 21.

With regard to the rejection relating to the anticipation and patentability by the references, the references indicated by the Examiner do not teach or suggest the technical features of the present invention. Accordingly, a skilled person would have had no reasonable expectation that PGCs originated from embryonic gonad, cultured for at least 10 days and separated from a media without using the process of Ficoll gradient centrifugation could successfully used for improving germline transmission of 44.7-49.7%, as recited in the present claims.

Therefore, in view of the foregoing amendments and remarks, the Applicants respectfully request reconsideration and the timely allowance of the pending claims.

If there are any charges not covered or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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Susan M. Michaud

Susan M. Michaud, Ph.D.
Reg. No. 42,885

Clark & Elbing LLP
101 Federal Street
Boston, MA 02110
Telephone: 617-428-0200
Facsimile: 617-428-7045